STATISTICAL APPENDIX

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The determination of the degree of potency of a serum is based upon the assumption that the different sera all contain one and the same agent, reagin, in different concentrations.

For each serum the dilution (F) giving a certain definite reaction is determined experimentally. This reaction is 60 per cent. haemolysis for the complement-fixation reactions and a flocculation degree of 2 in the Kahn reaction. Let C_0 denote the reagin concentration corresponding to this reaction; the concentration (C) of reagin in the serum to be examined is then determined by:

$$C = F \cdot C_0$$

or

$$\log C = \log F + \log C_0 .$$

The absolute potency of a serum, defined as the logarithm of F with $\frac{1}{3} \log 3$ as unit, is then given by:

$$\frac{\log F}{\frac{1}{3}\log 3} = \frac{1}{\frac{1}{3}\log 3} (\log C - \log C_0) .$$

Owing to variations in the blood employed and in the complement-fixation system, C_0 is not constant, but varies from day to day. What is determined in this way is therefore only an apparent potency or titre. However (as described by Schmidt, 1951) every day six standard sera are examined, for which a standard value has been determined in advance as the average titre for ten test days. The average difference in titre value from these standard values is then employed, each day, as a correction of the titres for the different test sera. Thus, the test sera are in fact measured on the basis of standard sera, so that it is a relative potency which is determined. Letting ξ denote the relative potency, we get:

$$\xi = \frac{1}{\frac{1}{3}\log 3} \log C - \frac{1}{\frac{1}{3}\log 3} \log C_0 + \text{corr.}$$

or

$$\xi = \alpha + \theta , \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where

$$\alpha = - \frac{1}{\frac{1}{3}\log 3} \log C_0 + \text{corr.},$$

and

$$\theta = \frac{1}{\frac{1}{3}\log 3} \log C .$$

In the Copenhagen laboratory this correction is in current use for complement-fixation reactions. For technical reasons a similar correction has not so far been applicable to the Kahn reaction, in which case, therefore, ξ in the formula (1) — with the correction = 0 — should be interpreted as an apparent potency only.

In practice, however, ξ can only be determined subject to a certain chance variation. The relation (1) therefore takes the form

$$x = \alpha + \theta + u \quad . \quad . \quad . \quad (2)$$

where u is a random variable with a certain standard deviation σ. As it will appear from the following, the order of magnitude of σ is 2. A value so large can only partly be explained by technical variations, as double determinations will not often show differences larger than 1 degree of potency. The remaining part of the variation is assumed to result from the presence in the different sera of certain agents which affect the reaction—possibly depending on the antigen—but are randomly distributed from one serum to the other. The standard deviation σ is assumed to be independent of the potency, a working hypothesis which will be tested subsequently.

Assuming that it is the same reagin that reacts with the different antigens, and letting x_ν, y_ν, and z_ν denote the potencies of the ν–th serum for WRM, CWRM, and KR, respectively, the following specification is obtained:

$$\left.\begin{aligned} x_\nu &= \alpha_1 + \theta_\nu + u_{\nu 1} \\ y_\nu &= \alpha_2 + \theta_\nu + u_{\nu 2} \\ z_\nu &= \alpha_3 + \theta_\nu + u_{\nu 3} \end{aligned}\right\} \quad . \quad . \quad . \quad (3)$$

where $u_{\nu 1}$, $u_{\nu 2}$, and $u_{\nu 3}$ are quantities varying randomly about 0 with variances σ_1^2, σ_2^2, and σ_3^2. The u's are furthermore assumed to be stochastically independent.

The specification set up above is clearly a simplification of actual conditions. The model has been used in an investigation of whether the relations between the potencies of the three antigens are the same for the three clinical groups.

By means of the equations (3) the dependence between, for example, x and z can be established. We find that:

$$x_\nu = \alpha_1 - \alpha_3 + z_\nu + u_{\nu 1} - u_{\nu 3} .$$

For a fixed value of z, the variance of x will be:

$$\sigma^2_{x|z} = \sigma_1^2 + \sigma_3^2 \quad . \quad . \quad . \quad . \quad (4a)$$

which, in turn, is equal to $\sigma^2_{z|x}$. Corresponding

relations can be found for the other combinations :

$$\sigma^2_{y|x} = \sigma^2_1 + \sigma^2_3 = \sigma^2_{x|y} \quad . \quad . \quad (4b)$$

and

$$\sigma^2_{y|z} = \sigma^2_2 + \sigma^2_3 = \sigma^2_{z|y} \quad . \quad . \quad (4c)$$

In carrying out the analysis we are faced with the difficulty that the measurements do not give us any results smaller than zero. If there is no reagin in a serum, the potency should be $-\infty$; and for sera with a low reagin content the potency should be negative. The 0-values observed accordingly represent sera with no reagin content as well as sera, the reagin contents of which are too low to give a reliable positive reaction. Fig. 1, illustrating a two-dimensional distribution for one of the clinical groups, shows clearly an accumulation of (0, 0)-values. In the distribution of one variable for given positive value of another variable, sera containing no reagin should not be comprised. These conditional distributions have been investigated by the probit method (see Fisher and Yates, 1943 ; Arley and Buch, 1950, § 19, 7) ; it was found that they can be regarded, with good approximation, as normal distributions, however in some cases truncated at zero. That is to say, the number of 0-values in the individual distributions might be imagined to be distributed over the range of negative values in such a manner as to make the distributions normal.

For each distribution is made an estimate of the mean value, written $\bar{x}$ (WR-M), $\bar{y}$ (C-WR-M), and $\bar{z}$ (KR), and an estimate of the variance, the method developed by Hald (1949), having been used in the case of the truncated distributions. Altogether, 9 × 2 sets of calculations have been made. A comparison of the estimated variances for each set indicated that the hypothesis of a constant variance was acceptable. Consequently, the individual variance estimates for each set have been pooled by calculating a weighted average. The estimates of mean value and variance being stochastically dependent in the case of the truncated distributions, improved estimates of the mean values have been calculated for these, taking advantage of the fact that the variance might be regarded as constant. The mean value estimates and the total variance estimates (s^2) found appear from Appendix Tables IA and IB. Table IA furthermore shows weight factors w, which for the non-truncated distributions are equal to the number of observations, and for the truncated distributions appear as the result of

APPENDIX TABLE IA

MEAN VALUE ESTIMATES KR, WR-M, AND C-WR-M AND WEIGHT FACTORS (w)

WR-M	SI				SIII				"Rest"			
	w	KR	w	C-WR-M	w	KR	w	C-WR-M	w	KR	w	C-WR-M
1	5	0·52	0	<0·00	11	1·16	12	3·50	14	1·02	13	0·29
2	6	−1·40	5	−2·51	4	1·16	4	1·11	14	2·57	14	3·00
3	6	1·20	4	−1·46	1	3·00	1	7·00	11	3·09	11	3·82
4	8	−0·36	0	<0·00	5	0·65	6	4·00	21	3·52	22	3·82
5	12	0·18	9	−0·18	2	0·05	2	8·00	10	4·20	10	5·60
6	6	1·67	5	0·44	2	6·00	2	5·50	8	4·62	8	5·75
7	6	3·17	6	1·58	—	—	—	—	10	6·00	10	7·50
8	9	4·67	9	5·22	2	6·00	2	8·00	8	7·75	8	7·75
9	1	6·00	1	3·00	—	—	—	—	5	8·40	5	8·00
10	1	9·00	1	8·00	—	—	—	—	2	9·00	2	8·50
11	1	3·00	2	7·00	—	—	—	—	1	10·00	1	11·00
KR	w	C-WR-M	w	WR-M	w	C-WR-M	w	WR-M	w	C-WR-M	w	WR-M
1	13	−1·49	21	4·62	17	1·05	21	0·50	26	0·05	26	0·06
2	5	−0·35	7	4·86	5	3·40	4	0·02	18	2·67	18	2·89
3	5	4·40	5	6·40	4	1·90	6	1·83	12	3·42	12	2·33
4	2	0·00	3	7·00	—	—	—	—	17	4·12	17	4·00
5	5	4·40	5	6·20	2	6·50	2	7·00	11	4·82	11	4·82
6	1	3·00	1	9·00	—	—	—	—	12	7·25	12	5·42
7	2	6·50	2	7·50	2	7·00	2	7·00	8	7·50	8	7·25
8	—	—	—	—	—	—	—	—	2	9·00	2	8·00
9	3	8·00	3	10·49	—	—	—	—	7	8·00	7	8·71
10	0	>12·00	0	>12·00	—	—	—	—	4	9·00	3	11·69
11	1	8·00	1	8·00	—	—	—	—	3	9·98	3	9·98
C-WR-M	w	WR-M	w	KR	w	WR-M	w	KR	w	WR-M	w	KR
1	9	4·89	8	1·50	2	−1·94	4	1·02	5	1·80	5	2·20
2	5	4·80	5	2·17	7	−0·66	8	−0·14	20	2·10	20	2·05
3	3	6·67	3	3·00	3	1·12	3	2·20	13	3·54	13	2·69
4	1	5·00	0	<0·00	4	1·22	4	1·50	6	2·18	6	2·33
5	5	8·00	5	3·60	6	3·00	6	2·17	23	4·87	23	4·13
6	—	—	—	—	2	3·50	2	4·50	4	3·25	3	4·33
7	—	—	—	—	2	2·00	2	−0·14	9	5·89	9	6·44
8	8	8·88	8	7·25	5	6·00	4	3·36	14	7·50	14	7·78
9	—	—	—	—	—	—	—	—	10	8·30	10	7·40
10	—	—	—	—	—	—	—	—	1	8·00	1	7·00
11	—	—	—	—	—	—	—	—	1	11·00	1	10·00

APPENDIX TABLE IB

TOTAL VARIANCE ESTIMATES

(Parenthesized figures denote sum of weights for separate estimates)

	SI	SIII	" Rest "
$s^2_{y.x}$	8·49 (23)	5·87 (21)	3·28 (81)
$s^2_{x.y}$	5·25 (25)	6·52 (19)	3·55 (90)
Average	*6·87* (24)	*6·20* (20)	*3·42* (85)
$s^2_{y.z}$	6·22 (17)	9·08 (23)	2·96 (94)
$s^2_{z.y}$	4·79 (23)	2·40 (22)	2·90 (82)
Average	*5·50* (20)	*5·74* (22)	*2·93* (88)
$s^2_{x.z}$	5·93 (39)	3·14 (18)	3·39 (79)
$s^2_{z.x}$	5·24 (40)	1·83 (18)	3·50 (85)
Average	*5·58* (40)	*2·48* (18)	*3·45* (85)

reducing the number of observations by a certain factor depending on the degree of truncation.

Letting $\theta^{(i)}$ denote the mean of the θ-values for those sera, for which KR has a certain value $z^{(i)}$, we have the following relations :—

$$\left.\begin{aligned} E(\bar{x}^{(i)}) &= \alpha_1 + \theta^{(i)} \\ E(\bar{y}^{(i)}) &= \alpha_2 + \theta^{(i)}, \end{aligned}\right\} \quad . \quad . \quad (5)$$

where $\bar{x}^{(i)}$ and $\bar{y}^{(i)}$ are the estimated mean values of the distributions. The difference between the mean value estimates,

$$d^{(i)} = \bar{y}^{(i)} - \bar{x}^{(i)} \approx \alpha_2 - \alpha_1, \quad . \quad (6)$$

must consequently be independent of the θ-values as well as of $z^{(i)}$. These relations are, however, only approximatively valid for the truncated distributions.

The variance of $d^{(i)}$ is, in the cases where there is no truncation, determined by the expression

$$\mathrm{Var}\,(d^{(i)}) = \frac{\sigma_2^2 + \sigma_1^2}{w^{(i)}} \quad . \quad . \quad (7)$$

Where $w^{(i)}$ is the number of observations as given by the two identical weight factors w in Table IA. In the case of the truncated distributions, the two weight factors are not quite equal ; as approximation has then been used :

$$w^{(i)} = \frac{1}{2}(w_1^{(i)} + w_2^{(i)}) \quad . \quad . \quad (8)$$

As estimated value of $\sigma_2^2 + \sigma_1^2$ is inserted the average value of the total variance estimate as for the calculations of x for given y and of y for given x (Table IB).

The results of the calculations of the d-values appear from Table II, in which also the weights (w) are stated.

For the purpose of comparing WR-M/C-WR-M, clinical group SI, the "mean-value-estimates" corresponding to the given values of KR are plotted with

APPENDIX TABLE II

THE DIFFERENCES BETWEEN " MEAN VALUE ESTIMATES " OF TWO OF THE REACTIONS FOR GIVEN VALUE (i) OF THE THIRD REACTION

	$\overline{KR} - \overline{C\text{-}WR\text{-}M}$						$\overline{C\text{-}WR\text{-}M} - \overline{WR\text{-}M}$						$\overline{WR\text{-}M} - \overline{KR}$					
	SI		SIII		" Rest "		SI		SIII		" Rest "		SI		SIII		" Rest "	
	d	w	d	w	d	w	d	w	d	w	d	w	d	w	d	w	d	w
1	—	—	−2·34	11·5	0·73	13·5	−6·11	17·0	0·55	19·0	−0·01	26·0	3·39	8·5	−2·96	3·0	−0·40	5·0
2	1·11	5·5	0·05	4·0	−0·43	14·0	−5·21	6·0	3·38	4·5	−0·22	18·0	2·63	5·0	−0·52	7·5	0·05	20·0
3	2·66	5·0	−4·00	1·0	−0·73	11·0	−2·00	5·0	0·07	5·0	1·09	12·0	3·67	3·0	−1·08	3·0	0·85	13·0
4	—	—	−3·35	5·5	−0·30	21·5	−7·00	2·5	—	—	0·12	17·0	—	—	−0·28	4·0	−0·15	6·0
5	0·36	10·5	−7·95	2·0	−1·40	10·0	−1·80	5·0	−0·50	2·0	0·00	11·0	4·40	5·0	0·83	6·0	0·74	23·0
6	1·23	5·5	0·50	2·0	−1·13	8·0	−6·00	1·0	—	—	1·83	12·0	—	—	−1·00	2·0	−1·08	3·5
7	1·59	6·0	—	—	−1·50	10·0	−1·00	2·0	0·00	2·0	0·25	8·0	—	—	2·14	2·0	−0·55	9·0
8	−0·55	9·0	−2·00	2·0	0·00	8·0	—	—	—	—	1·00	2·0	1·63	8·0	2·64	4·5	−0·28	14·0
9	3·00	1·0	—	—	0·40	5·0	−2·49	3·0	—	—	−0·71	7·0	—	—	—	—	0·90	10·0
10	1·00	1·0	—	—	0·50	2·0	—	—	—	—	−2·69	3·5	—	—	—	—	1·00	1·0
11	−4·00	1·5	—	—	−1·00	1·0	0·00	1·0	—	—	0·00	3·0	—	—	—	—	1·00	1·0
Σw	45·0		28·0		104·0		42·5		32·5		119·5		29·5		32·0		105·5	
$\overline{d}$	0·72		−2·43		−0·45		−4·40		0·77		0·19		2·98		0·06		0·23	
SS_d	80·20		111·31		51·23		189·13		38·43		82·69		28·10		78·65		36·07	
f_d	8		6		10		8		4		10		4		7		10	
$\frac{SS_d}{f_d}$	10·03		18·55		5·12		23·64		9·61		8·27		7·03		11·24		3·61	
"χ" ^{2}	1·82		3·25		1·77		3·43		1·55		2·43		1·26		4·50		1·03	
P in per cent.	10–30		1–2·5		5–10		0·5–1		10–30		1–2·5		30–50		0·1–0·5		30–50	
ME_d	0·53		0·67		0·24		—		0·76		0·24		0·64		—		0·26	

$\overline{\text{WR-M}}$ as ordinates and $\overline{\text{C-WR-M}}$ as abscissae; corresponding diagrams for the other comparisons are given in Figs 2–10.

If the $d^{(i)}$ varies at random about the α-difference with a variance determined by Expression (7), the sum of squares

$$SS_d = \sum_{i=1}^{k} (d^{(i)} - d^{(\cdot)})^2 w_i, \text{ where } d^{(\cdot)} = \frac{\Sigma d^{(i)} w_i}{\Sigma w_i},$$

must follow a $\chi^2(\sigma_1^2 + \sigma_2^2)$-distribution with k-1 degrees of freedom. Division of the sum of the squares by k-1 should accordingly lead to an estimate of $\sigma_1^2 + \sigma_2^2$. If this estimate differs significantly from the one previously found, the hypothesis is rejected.

The ratio (v^2) between the two estimates is stated in Table II. By means of a v^2-table (Hald, 1952), the probability P of obtaining a v^2-value greater than or equal to the observed value has been found. As these P-values are only approximate, it will be doubtful whether there is actual significance in the cases where the P-value found is close to the limit of significance, which is usually fixed at a P of 5 per cent. A significant v^2-value can appear as a result either of systematic non-parallelism with the identity line, or of too great a variation without any dependence upon the level of potency.

It will appear from Table II that all the P-values are rather low. With the exception of the comparisons C-WR-M/WR-M in SI and WR-M/KR in SIII, where the deviation is of a systematic nature, it seems that only random variations of the differences are met with. This means that a certain extra variation of the $d^{(i)}$'s must be taken into consideration in order to avoid underestimating the standard errors of the average differences.

Supposing that this extra variation is caused by the α-differences varying randomly from one i-value to the other with a certain standard deviation τ, we find, as for the comparison C-WR-M/WR-M, that

$$\text{Var}(\bar{d}) = \frac{1}{\Sigma w_i}\left(\sigma_1^2 + \sigma_2^2 + \tau^2 \frac{\Sigma w_i^2}{\Sigma w_i}\right) \quad . \quad (10)$$

An estimate of τ^2 can be obtained from MS_d, since

$$E(MS_d) = \sigma_1^2 + \sigma_2^2 + \frac{\tau^2}{k-1}\left(\Sigma w_i - \frac{\Sigma w_i^2}{\Sigma w_i}\right), \quad (11)$$

Whence

$$\tau^2 \approx \frac{MS_d - \frac{1}{2}(s_{x|y}^2 + s_{y|x}^2)}{\frac{1}{k-1}\left(\Sigma w_i - \frac{\Sigma w_i^2}{\Sigma w_i}\right)} \quad . \quad . \quad (12)$$

Corresponding formulae hold good for the other comparisons. Calculations of estimates of τ^2 show the latter to be about 1·0 for SI and SIII and about 0·25 for the "Rest". Introduction of these estimates and the estimates of $\sigma_1^2 + \sigma_2^2$ in (10) and the corresponding formulae leads to estimates of the variances of the $\bar{d}$'s.

It is investigated, for group SI, whether the difference between C-WR-M and WR-M depends on the level of potency. Average difference estimates are calculated for KR = 1–4 and KR $\geqslant$ 5. The results are as follows:

KR	$\bar{d}$	Var($\bar{d}$)	SE($\bar{d}$)
1–4	−5·33	0·61	0·78
5–11	−2·04	0·85	0·92

Difference −2·04 − (−5·33) = 3·29
Standard Error of Difference $\sqrt{0{\cdot}61 + 0{\cdot}85}$ = 1·21

These two average figures are significantly different. The difference is 2·7 times the standard error. The difference between the potencies obtained with the two antigens is consequently dependent on the level of potency. As for the two other comparisons within this group, the hypothesis of constant difference is acceptable, and the average difference between WR-M and KR is significantly different from 0.

In the case of group SIII, the hypothesis of constant difference is not acceptable for the comparison WR-M/KR. Calculating the average difference estimates for C-WR-M = 1–4 and C-WR-M $\geqslant$ 5, we find:

C-WR-M	$\bar{d}$	Var($\bar{d}$)	SE($\bar{d}$)
1–4	−0·98	0·43	0·66
5–11	1·32	0·48	0·69

Difference 1·32 − (−0·98) = 2·30
Standard Error of Difference $\sqrt{0{\cdot}43 + 0{\cdot}48}$ = 0·95

In this case, too, a dependence upon the level of potency can be established. The difference is 2·4 times the standard error.

As regards the comparison C-WR-M/WR-M, there are very few observations for KR $\geqslant$ 5. Calculating the average difference for KR = 1–4, we get:

KR	$\bar{d}$	Var($\bar{d}$)	SE($\bar{d}$)
1–4	0·91	0·22	0·47

This value is not significantly greater than 0, but it is very clearly different from the corresponding value found for group SI.

In this paper only the quantitative analysis has been dealt with. In the qualitative analysis the important question is whether or not the reaction is positive. The probability that a reaction is greater than zero is a function of the ratio between mean potency ($\alpha + \theta$) and standard deviation (σ), namely,

$$\varphi\left(-\frac{\alpha+\theta}{\sigma}\right)$$

where $\varphi(x)$ denotes the area function of the normal distribution. If the mean potencies are the same for the two reactions while the standard deviations are different, the greatest number of 0-values will be found in the case of that reaction for which the standard deviation is greatest.

Conclusion

As a result of the investigation the following deviations from the model formulated have been established :

(1) The differences are not the same in the three clinical groups.

(2) In some groups the difference is dependent on the level of potency.

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